BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *VICIA FABA* AND THEIR ANTIMICROBIAL STUDIES

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ABSTRACT

Nanoscale Silver particles have biological applications because of its unique properties, antimicrobial activity. The aim of the work is to test effects of Silver nanoparticles from *Vicia faba* using suggested bacterial colonies. Biosafety and biocompatibility tests for Nanoparticles and conjugate with *Vicia faba* are preformed. UV-visible spectroscopy is an important technique for analyzing the formation of silver nanoparticles in aqueous solution. AgNPs has free electron, which gives rise to plasma resonance absorption band, due to combined vibration of metal nanoparticles in resonance with the light wave. The presence of the elemental silver can be seen in the graph presented by EDX, which indicates the reduction of silver ions to elemental silver. Screening of ethanolic extracts of *Vicia faba* shows moderate antibacterial activities against different bacteria. The silver nanoparticles of average size have been synthesized using dried leaves of plant *Vicia faba*. Characterization from UV-Vis, FT- IR, SEM, EDX support the stability of biosynthesized nanoparticles. The silver nanoparticles using *Vicia faba* exhibit excellent antibacterial activity.

Key words: Nano medicine, Silver nanoparticle, *Vicia faba*.

INTRODUCTION

Nanotechnology is an enormously powerful technology, which holds a huge promise for the design and development of many types of novel products with its potential medical applications on early disease detection, treatment, and prevention. Nanoparticles, because of their small size, have distinct properties compared to the bulk form of the same material, thus offering many new developments in the fields of biosensors, biomedicine, and bio nanotechnology. Silver is the metal of choice as they hold the promise to kill microbes effectively. Silver nanoparticles have been recently known to be a promising antimicrobial agent that acts on a broad range of target sites both extracellular a well as intracellular. Silver is widely used as a catalyst for the oxidation of methanol to formaldehyde and ethylene to ethylene oxide [1-7]. Ag NP highly antimicrobial to several species of bacteria, including the common kitchen microbe, *E. coli*. According to the mechanism reported, silver nanoparticles interact with the outer membrane of bacteria, and arrest the respiration and some other metabolic pathway that leads to the death of the bacteria. Nano-sized silver havem already provides a more durable antimicrobial protection, often for the life of the product. In medicines, silver and silver nanoparticles have a ample application including skin ointments and creams containing silver to prevent infection of burns and open wounds, medical devices and implants prepared with silver-impregnated polymers. In textile industry, silver-embedded fabrics are now used in sporting equipment. The term "nanoparticles" is used to describe a particle with size in the range of 1nm-100nm, at least in one of the three possible dimensions. In this size range, the physical, chemical and biological properties of the nanoparticles changes in fundamental ways from the properties of both individual atoms/molecules and of the corresponding bulk materials [8-13].

The present work is aimed to develop a novel approach for the green synthesis of silver nanoparticles using extract of V*icia faba*. To analyses the primary and secondary metabolites present in the plant *Vicia faba* in qualitatively and quantitatively. *Vicia faba* belongs to fabaceae family. It yield broad beans, have a long tradition of cultivation. It grows well in nitrogen poor soil.

MATERIALS AND METHODS

Preparation of *Vicia faba* Leaves Extract

The fresh *Vicia faba* leaves were collected from Peruvallanallur, in Trichy district. The leaves were thoroughly washed several times using normal water and then followed by distilled water to remove impurities. The cleaned leaves were subsequently dried under sunshade to remove moisture completely, powdered by using mechanical grinder and then stored. The 20 g of powdered plant leaves were taken into a beaker along with 100 ml of ethanol and the leaves were soaked ethanol at room temperature. The prepared solution was initially filtered through normal filter paper thereby powdered leafy materials will be filtered out. The filtrate was again filtered through Whatman No.1 filter paper to get clear solution. The filtrate was stored at 4°C for future works [14-18].

Phytochemical Screening

The Phytochemical Screening tests were carried out by standard procedures.

SYNTHESIS OF SILVER NANO PARTICLES

Plant Extraction

The dried plant powder was pulverized well with mortar and pestle to make a powder. Twenty grams of powder sample was mixed into 100 ml of deionized water and the mixture was boiled for 10 min. After cooling the leaf extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4°C for further use.

Synthesis of Silver nanoparticles using plant extract:

For the Ag nano particle synthesis, 5 ml of *plant extract* was added to 45 ml of 1 mM aqueous $AgNO_3$ solution in a 250 ml Erlenmeyer flask. The flask was then incubated in the dark at 5hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without plant extract. The Ag nanoparticle solution thus obtained after five hours was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water for further use.

CHARACTERIZATION OF SILVER NANO PARTICLES

UV-Visible Analysis

The extracts were examined under visible UV-Visible spectrum. The sample is dissolved deionized water. The Nanoparticles were scanned in the wavelength ranging from 190-1100nm using Systronic Spectrophotometer. These solutions were scanned in turn at intervals of 50 nm and the characteristic peaks were detected. The peak value of the UV-Visible was recorded.

Fourier Transform Infrared (FT-IR) Spectroscopic Analysis

Spectra were obtained with the aid of an OMNI-sampler attenuated total reflectance (ATR) accessory on a FTIR spectrophotometer (Perkin Elmer Spectrophotometer system, USA) followed by previous methods with some modification (Liu *et al*., 2006). A small amount of sample was respectively placed directly on sample holder of the infrared spectrometer with constant pressure applied and data of infrared absorbance, collected over the wave number ranged from 4000 cm^{-1} to 400 cm^{-1} and computerized for analyses by using the 21 CFR part 11 software. The reference spectra were acquired from the cleaned blank crystal prior to the presentation of each sample replicate. The peak values of FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum.

SEM analysis of silver nanoparticles

Scanning electron microscopic (SEM) analysis was done using VEGA3 LMU machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the Silver nanoparticles on the grid. Extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

EDX spectrum

The chemical composition of bio synthesized silver nano particles was revealed by EDX spectrum.

DETERMINATION OF ANTIMICROBIAL ACTIVITY

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka et al., 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Escherichia coli*, *Staphylococcus auerus* were spread on Nutrient agar plates for bacteria and *Candida albicans* and *Aspergillus flavus* were spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl, 100 µl and 150 µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria and at room temperature (30 ± 1) for 24-48 hr. for fungal strains. Each sample was tested in triplicate.

RESULTS AND DISCUSSION

UV-visible spectroscopy

UV-visible spectroscopy is important technique for analyzing the formation of silver nanoparticles in aqueous solution. Silver nanoparticles, which gives rise to plasma resonance absorption band, due combined vibration of metal nanoparticles in resonance with the light wave. A surface plasma resonance spectrum of silver nanoparticle was obtained at 437nm after 5min color changing to brownish color [14-17]. In figure the surface plasma silver nanoparticles at increasing concentration was taken and the color changes were observed for silver nanoparticles .For silver color changes from colorless to brownish color. Metal nanoparticles can be synthesized by reducing metal ions using some chemical molecules .In green synthesis ,it is observed that natural material extract act as reducing agent for generation of metal nanoparticles.

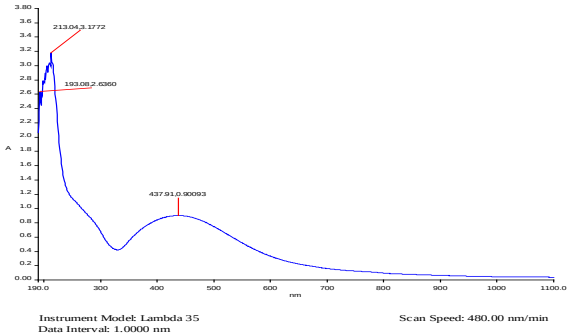


Fig. -4.2: UV-Vis spectrum of Silver Nanoparticles

FT-IR spectroscopy

The FT-IR spectrum of silver nanoparticles is shown in fig 4.5. The Infra Red spectrum of silver nanoparticles shown bands at 3434.72 $cm^{-1}$and 3466.20cm^{-1} (phenolic O-H stretching vibration). The peaks at 2832.15 cm-1 indicates C-H stretching of methlene group. The bands at 1630.98 cm-1 states primary amines. The bands at 1363.07 cm-1 state C-H rock alkenes and bands at 1016.32cm-1 indicates the presence of C-O stretching of alcohols, carboxylic acids, esters and ethers. An reduction of silver ions in the present investigation might have resulted due to water soluble phytochemicals like flavanones, quinones and organic acids present in the leaves of *Vicia faba*, silver reduction due to phytochemicals (flavonoids or other poly phenols), some proteins and metabolites such as terpenoids having functional group of alcohols, ketons, aldehydes and carboxylic acid present in *Vicia faba* leaves may be considered as the significant observed in this direction. Based on the FT-IR analysis it is confirmed that the broad peaks of phenols and proteins acts as a reducing, stability and capping agents and for silver nanoparticles from the state of silver radical to silver ions.

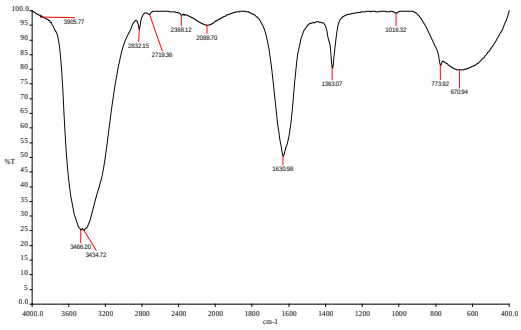


Fig. -4.3: FT-IRspectrum of Silver Nanoparticles

Scanning Electron Microscopy (SEM)

Thin films of the sample were prepared on clean glass slide by just dropping a very small amount of the sample and extra solution was removed by using a blotting paper and then the film was allowed to dry for 10 min. in Hot air oven. For conventional imaging in the SEM Analysis biological or insulating samples require thin conductive coating. The surface of the thin films is coated by gold acts as a electrically conductive agent. SEM analysis shows uniform distribution of silver nanoparticles on the surfaces with spherical shape with particle size range from 27 nms to 105 nms. The SEM result shown in figure [4.6].

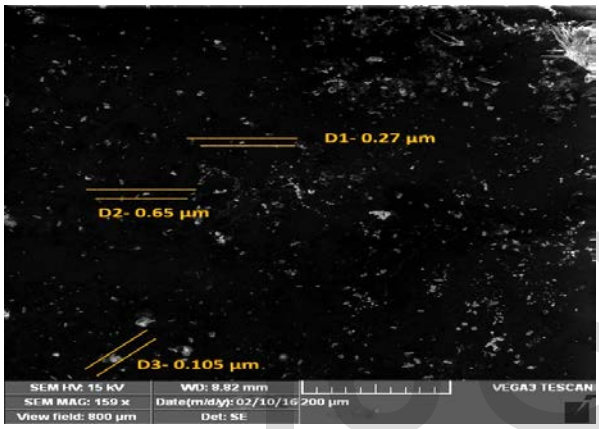
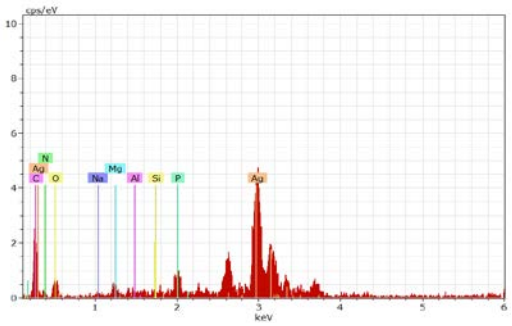


Fig. 4.4. High resolution scanning electron microscopic (SEM)
(Polydispersed AgNPs ranged between 27–105nm)

EDAX analysis

The presence of the elemental silver can be seen in the graph presented by EDAX, which indicates the reduction of silver ions to elemental silver ions, and EDAX analysis was performed to knowing percentage of silver nanoparticles in the sample. For this the synthesized silver nanoparticles was characterized by using FEI Quanta 200 FEG HR-SEM equipped with EDAX instrument. The EDAX spectra shows the different types of elements with their weight percentage like Carbon (6.94) Nitrogen (5.14) Oxygen (7.13), Sodium (0.24), Magnesium(0.24), Aluminum (0.09), Silicon (0.04), Silver (0.95) and Phosphorous(0.19) in the sample



El AN Series unn. C norm. C Atom. C Error (1 Sigma)

			[wt.%]	[wt.%]	[at.%]	[wt.%]
C	6	K-series	20.51	31.51	51.54	6.94
O	8	K-series	13.74	21.12	25.93	7.13
N	7	K-series	5.73	8.81	12.36	5.14
Ag	47	L-series	21.43	32.93	6.00	0.95
P	15	K-series	1.52	2.34	1.48	0.19
Mg	12	K-series	1.19	1.83	1.48	0.24
Na	11	K-series	0.74	1.14	0.97	0.24
Al	13	K-series	0.19	0.29	0.21	0.09
Si	14	K-series	0.03	0.04	0.03	0.04

ANTIMICROBIAL ACTIVITY

Bio synthesized silver nanoparticles were analyzed for their antimicrobial activity against two bacterial species like *Escherichia coli* (ESBL-3984), *Staphylococcus aureus* (MTCC 96),the result are shown in the table [4.7]. Antifungal studies are taken out from two fungal species like *Candida albicans* (MTCC 227) *and* Aspergillus *flavus* (MTCC 230) [18-23].

The results are shown in Table 4.5. The obtained results are indicative of the diameter of zone of inhibition due to microbial susceptibility. The synthesized silver nano particles shows higher inhibiting effect on *E.coli* (4.52) followed by *Staphylococcus aureus* (4.21).

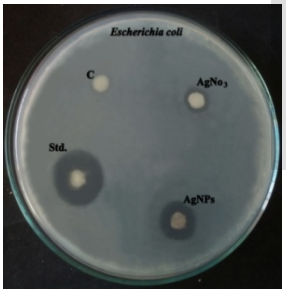
Table 4.5. Antimicrobial activity of Silver nanoparticles

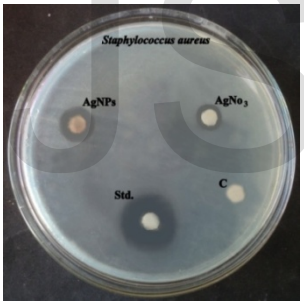
Microorganisms	$AgNO_3$ (µl)	AgNPs (µl)	Standard (µl)	Control (solvent) (µl)
Escherichia coli (mm)	0.48±0.05	4.52±0.31	7.62±0.53	0
Staphylococcus aureus (mm)	0.35±0.04	4.21±0.29	7.35±0.51	0
Candida albicans (mm)	0.27±0.02	3.76±0.26	7.19±0.49	0
Aspergillus niger (mm)	0.14±0.01	3.09±0.21	6.84±0.47	0

Values were expressed as Mean ± SD

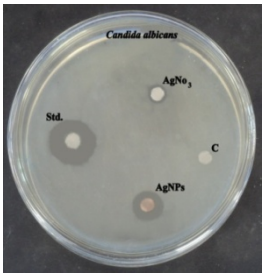
N.B. Negative Control = 0 Positive Control Bacteria - Chloromphenical
Positive Control Fungi – Fluconazole

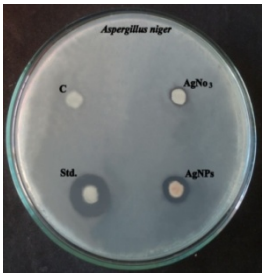
Escherichia coli ***Staphylococcus aureus***





Candida albicans ***Aspergillus niger***





CONCLUSION:

In this study we have developed eco friendly and environment safe green method for the synthesis of silver of silver nanoparticles from V*icia faba* leaves extract with rapid speed. The leaves extract very much suitable for the synthesis of small sized silver nano particles. The colour changes from light brown to dark brown indicates the presence of different phytochemicals responsible for the reduction, stabilization and capping of silver nanoparticles, which is confirmed by UV-Vis spectroscopy and FT-IR.FT-IR reveals that the phenols and primary amines of proteins are mainly responsible for the reduction and capping of this nanoparticles to prevent agglomeration and provide stability to the medium. The nanoparticles are very small in range between 27-105nms confirmed by SEM and analysis of total content silver nanoparticles by EDAX instrument. Further the antimicrobial studies indicated that the nanoparticles are toxic to different types to dry resistant micro organisms. Finally we conclude that the leaves of V*icia faba* are ideal material for the rapid synthesis of silver nanoparticles and act as a potential antimicrobial agent.

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